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REVERSAL OF NICOTINE ACTION ON THE INTESTINE BY ATROPINE

BY

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Evidence for the existence of an intrinsic inhibitory innervation in the wall of the intestine has been presented previously (Ambache, 1951). From experiments with botulinum toxin it could be deduced that the postganglionic neurones in the myenteric plexus are of two sorts, one set being motor and probably cholinergic, the other inhibitory and perhaps adrenergic (see Fig. 12 of the earlier paper). Botulinum toxin reversed the response to nicotine by producing a selective paralysis of the motor neurones, which allowed the effect of nicotine-stimulation of the inhibitory ganglia to be revealed.

Although the actions of atropine and of botulinum toxin at cholinergic nerve-endings are entirely different, yet, since atropine can also discriminate between cholinergic- and adrenergic-fibre effects, it was thought that reversal of nicotine action should be obtainable with atropine as with botulinum toxin. In preliminary experiments on rabbit's intestine, which are reported at the end of this paper, this expectation was not fulfilled. However, it was clear from these experiments that reversal could not be detected because of the persistence in the rabbit gut of the motor response to nicotine even after such high concentrations of atropine as 10^{-4}. But in preparations of intestine and of the gastric muscle from kittens it was possible to eliminate the motor response to nicotine with concentrations of atropine as low as 10^{-7} and to detect the occurrence of nicotine-reversal under conditions similar to those in which McSwiney and Robson (1929) observed "vagal reversal." An analogy between these two reversals produced by atropine is drawn in the discussion.

METHODS

Three adult rabbits (2.1–3.2 kg.) and six kittens, 8–12 weeks old (0.3–0.82 kg.), were killed by concussion. A segment of ileum was excised and suspended at once, with the lumen empty but closed at either end, in a 10-c.c. organ-bath full of Mg-free Tyrode (1.1 per cent $NaHCO_3$) oxygenated with a gas mixture of 95 per cent O_2 and 5 per cent CO_2.

The stomach strips were taken from two other 10-week kittens, both 0.49 kg. The stomach was removed and freed from omentum; after its contents had been washed out, the organ was slit open longitudinally, and the stomach wall was pinned down on a cork board with the mucosa facing upwards. An incision was made into the mucous membrane, and a plane of separation was found between it and the underlying muscle; the mucosa was removed as a sheet by undercutting it with a scalpel in that plane. A

longitudinal strip of the muscle, about 3 cm. long, was taken from the lesser curvature in one experiment, and from the greater in the other.

The test doses of nicotine were left in the bath for 30–60 seconds. The dosage refers not to the base but to the weight of the hydrogen tartrate salt. Hexamethonium was used as the di-bromide or di-iodide ; solutions of these were made from the powder.

RESULTS

Interaction of atropine and nicotine in kittens

Ileum.—Motor responses to 50–100 μg. of nicotine were present in five out of six preparations ; in one of these the response to nicotine was a mixture of contraction and inhibition. The nicotine-contractions were repeatable if the test doses were spaced 4–5 minutes apart. This action of nicotine was in all five experiments reversibly abolished by concentrations of $1–5 \times 10^{-7}$ of atropine. In favourable circumstances, when tone and rhythmic activity were retained after atropine, an inversion of the nicotine response became manifest (Fig. 1) and could be obtained

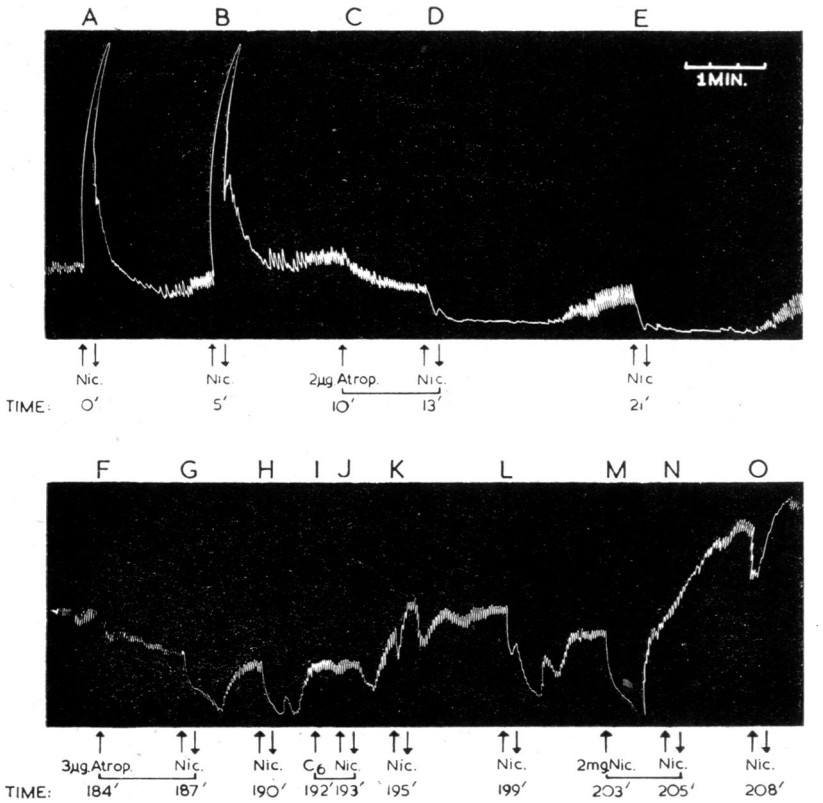

FIG. 1.—Kitten, 0.6 kg.; ileum preparation 40 cm. below pylorus, exhibiting inversion of the nicotine-response in the presence of, and after, atropine. Nicotine (50 μg.) left in the bath for 30 sec. at A, B, D, E, G, H, J, K, L, N, and O; 2 mg. for 120 sec. at M. Atropine (2 μg.) at C, and 3 μg. at F, introduced 3 min. before D and G respectively. Hexamethonium bromide (1 mg.) at I, 60 sec. before J. The minutes below, in this and subsequent figures, refer to the times of administration of each drug. For explanation see text.

repeatedly. Thus in four of the five experiments nicotine produced active inhibition of the gut, both in the presence of atropine and for some considerable time after it had been washed out (Fig. 2) ; eventually the gut recovered from the atropine

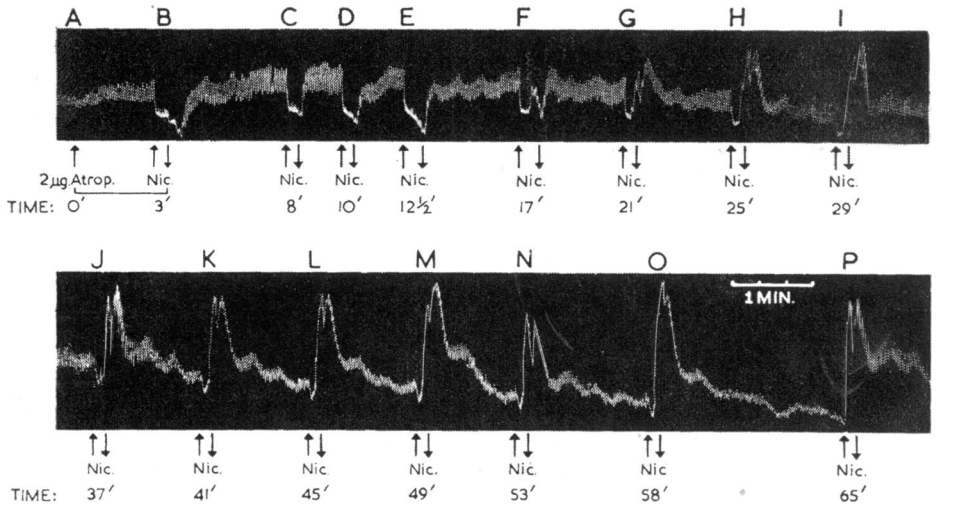

FIG. 2.—Kitten, 0.5 kg.; ileum preparation 20 cm. below pylorus. At A, 2 μg. atropine, left in the bath for 3½ min. At B, 50 μg. nicotine in the presence of atropine. During the next 65 min. at the times indicated, C–P, 50 μg. doses of nicotine, each left in the bath for 30 sec. (except E and F, 45 sec.). Note gradual decrease (after E) in size and duration of the inhibitory component, and growth of the motor component during recovery from atropine.

and the response to nicotine became motor, but could be reversed again with atropine. In the fifth experiment the gut relaxed, and small inhibitory responses to nicotine could be obtained only after the first two doses of atropine ; after the third dose of atropine the motor response to nicotine was abolished as usual, but there was little or no visible sign of inhibition owing to the already relaxed state of the gut.

In the sixth experiment, inhibitory responses to nicotine were recorded from the gut *without* atropinization ; 5–50 μg. nicotine produced a mixture of inhibition and contraction, but 100 μg. produced pure inhibition (Fig. 3).

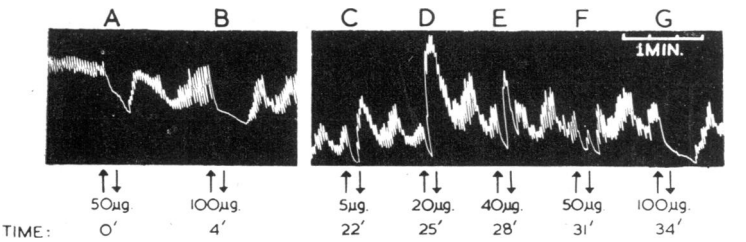

FIG. 3.—Kitten, 0.6 kg.; ileum preparation 29 cm. below pylorus, mounted 85 min. before A, but not subjected to any treatment during that interval. Inhibitory responses to nicotine *without* previous atropinization: the response to nicotine at A–G (each dose given for 30 sec.) is diphasic with 5–40 μg. (C, D, and E) but predominantly inhibitory with 50 μg. (A and F) and with 100 μg. (B and G).

As in the botulinized rabbit gut (Ambache, 1951) the inhibitory response to nicotine of the cat's ileum appears to be due to stimulation of inhibitory ganglion-cells because it is reversibly abolished after ganglionic block with 0.5–1 mg. hexamethonium salts or with 2 mg. nicotine (Fig. 1). As before, the observation was made that the inhibitory response to nicotine is antagonized by 1–2 mg. ephedrine, again suggesting that the inhibitory neurones concerned may be adrenergic.

Stomach strips.—The results of the experiments on the stomach strips from kittens confirm those on the ileum. This muscle is of interest because it was on it that McSwiney and Robson (1929) first demonstrated reversal by atropine of the effect of vagal stimulation in isolated nerve-muscle preparations. We are indebted to Prof. Robson for the information that this experiment, which is illustrated in Fig. 3 of their paper, was conducted entirely on preparations from cats ; the concentration of atropine which they used lay between 10^{-6} and 10^{-5}. Substituting ganglionic

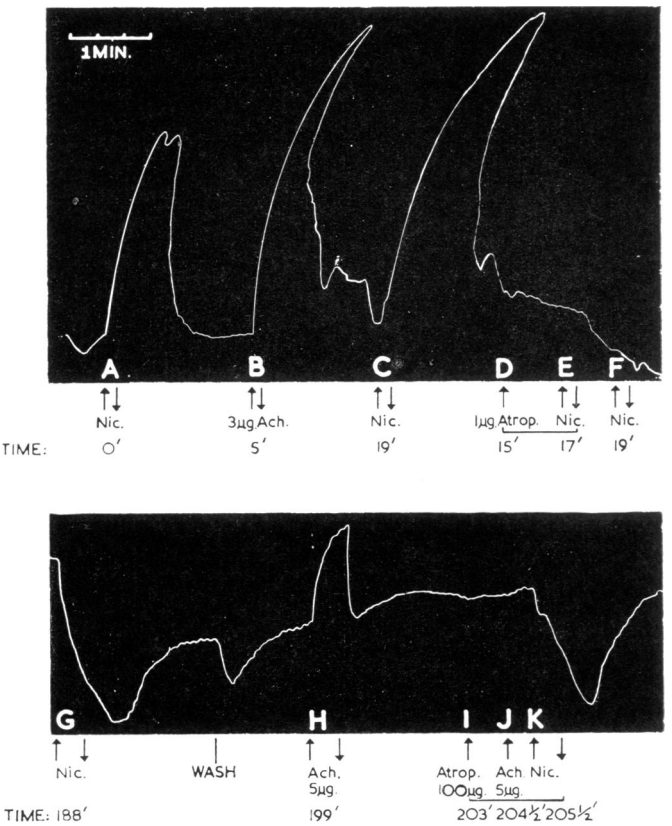

FIG. 4.—Kitten, 0.48 kg.; stomach strip from greater curvature, exhibiting nicotine-reversal after atropine. 200 µg. nicotine at A, C, E, and F, each for 30 sec.; and at G and K, each for 60 sec. Acetylcholine, 3 µg. at B, and 5 µg. at H and J. Atropine, 1 µg. introduced at D, 2 min. before the nicotine at E, and 100 µg. at I left in for 2½ min. till the end of K. The first dose of atropine at D abolished the motor response to nicotine, but nicotine-reversal was not marked until later. In the interval between F and G the muscle was treated several times with atropine, the last dose being 100 µg. atropine for 3½ min., 53 min. before G.

excitation by nicotine for the preganglionic stimulation used by McSwiney and Robson, we have been able to imitate pharmacologically the effect which they observed.

In the present experiments the stomach strips responded by contraction to 100–200 μg. nicotine; this response was abolished by atropine in concentrations of 10^{-7} in one experiment and 10^{-6} in the other. Nicotine-reversal was not detectable after the first treatment with atropine because of the relaxation of the strips, but after subsequent doses of atropine (1–100 μg.) tone was regained and nicotine-inversion was observed several times (Fig. 4). When present, the inhibitory effect of nicotine could be abolished by 1 mg. hexamethonium.

Non-reversal of the nicotine-response in atropinized rabbit's intestine

It was not possible to obtain a reversal of nicotine-action in rabbit's intestine because the motor response to nicotine persisted in the presence of even large amounts of atropine. As the dose of atropine was raised the response to nicotine diminished in size, but even at an atropine concentration of 10^{-4} remnants of nicotine-contraction were still present (Fig. 5). Pure inhibitory responses to nicotine were

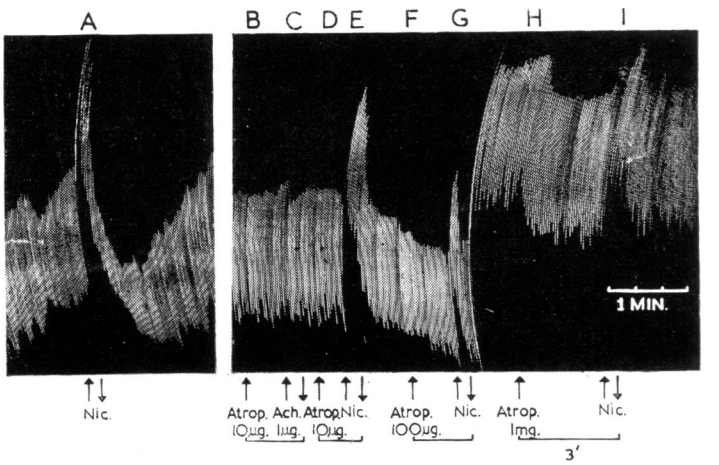

FIG. 5.—Rabbit, 2.15 kg.; ileum preparation, 30 cm. below pylorus, exhibiting persistence of motor response to nicotine in the presence of atropine (up to 10^{-4}). 50 μg. nicotine for 30 sec. at A, E, G, and I. Atropine, 10 μg. at B and D, 100 μg. at F, and 1 mg. at H (3 min. before the nicotine at I). Acetylcholine, 1 μg. at C. Interval of 110 min. between A and B; drum stopped between G and H, with change in baseline.

never recorded, although occasionally the nicotine-contraction was preceded by a loss of one or two beats in the spontaneous rhythm.

This result is based on 20 trials carried out in three different experiments, with atropine-concentrations ranging between 10^{-7} and 10^{-4}. In 15 of the trials the atropine preceded the nicotine by 30 seconds, in the other five by 3–4 minutes. The test dose of nicotine was 50 μg. in eight of the trials, in the other 12 it was reduced to 5 or 10 μg., as the gut was very sensitive. When low doses of nicotine were used, it was sometimes possible to abolish with atropine the contraction produced by 5 μg. nicotine, but reversal did not occur.

Discussion

The reversal of nicotine-action by atropine, like the reversal which is produced by botulinum toxin (Ambache, 1951), may be taken to indicate the presence in the gut of inhibitory postganglionic ("terminal") neurones, which respond to stimulation of their ganglion-cells by inducing relaxation of the gut. For a fuller discussion of the nature of these neurones the reader is referred to the earlier paper (page 60) in which a certain amount of evidence was presented for believing that these intrinsic inhibitor neurones may be adrenergic. It is only necessary to add that a migration of sympathetic neuroblasts from the neural crest to the prevertebral primordium and into the enteric plexus has been described by van Campenhout (1930) in an embryological study on frogs. The same primordium gives rise to the adrenal chromaffin tissue and to the ganglia of the sympathetic chain, i.e., to sympathin-containing nervous tissues. The *extrinsic* inhibitory fibres which the gut receives from the splanchnic nerves are known to be adrenergic; if it should be confirmed that the *intrinsic* inhibitors function by releasing the same transmitter, this identity between the two inhibitory innervations of the gut would provide a good example of economy in design.

In comparing the present results with those obtained previously on botulinized intestines the following agreements and discrepancies become apparent. Reversal of nicotine-action by atropine occurs in preparations from cats but not from rabbits. However, reversal of nicotine-action can occur in rabbits, but only after the motor nerve-endings in the gut have been paralysed with botulinum toxin; atropine (up to 10^{-4}) fails to reverse the action of nicotine because of the persistence, in its presence, of the motor effect of nicotine. Thus it is clear that atropine block of

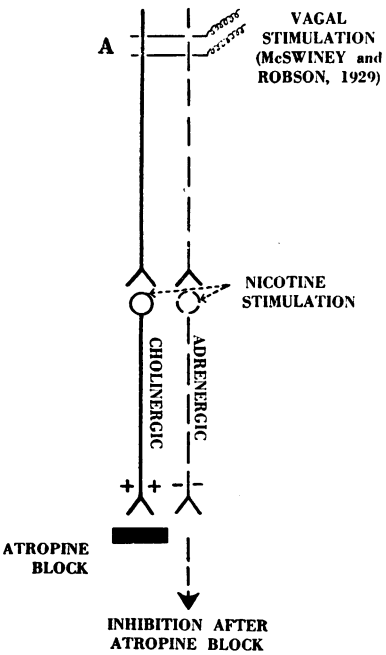

FIG. 6.—Diagram illustrating the possible analogy between vagal-reversal (Bayliss and Starling, 1899; McSwiney and Robson, 1929) and nicotine-reversal, after atropine.

the motor nerve-endings is for some reason effective in cats but not in rabbits; concerning other species little is known except that the nicotine-contraction of the guinea-pig gut is atropine-sensitive (Emmelin and Feldberg, 1947; confirmed by Ambache and Rocha e Silva, 1951, unpublished).

In considering the experiments on the stomach strips a useful parallel may be drawn between the reversal by atropine of the nicotine response, which we observed, and the effect found by McSwiney and Robson (1929) in isolated vagus nerve-stomach preparations. Their stimulus was applied to the preganglionic fibres (Fig. 6 at A), ours to the terminal ganglion-cells themselves. In both series of experiments the response was motor before, and inhibitory after, the atropine-block. If, following a suggestion first made by Langley (1922), we assume that the vagus links up with two distinct sets of postganglionic neurones, one motor (cholinergic) and the other inhibitory, then the analogy between vagal-reversal after atropine and nicotine-reversal becomes evident; this is set forth diagrammatically in Fig. 6.*

It would be of interest to repeat McSwiney and Robson's (1929) experiment on vagus-stomach preparations from rabbits; on the basis of the above analogy vagal-reversal by atropine would be expected to be, like nicotine-reversal, unobtainable in the rabbit because, as we have seen, the latter exhibits atropine-resistance, at least in the intestine. It is beyond the scope of this paper to discuss the nature of this resistance, for which the reader is referred to page 141 of Dale and Gaddum's paper (1930), but the present experiments do show that it is a matter subject to species-variations.

SUMMARY

1. In isolated intestinal preparations, and in stomach strips taken from young kittens, atropine-concentrations of $1–5 \times 10^{-7}$ abolish nicotine-contractions and reveal an inhibitory action of nicotine. This action is ganglionic, since it is abolished by hexamethonium; it is also abolished by ephedrine ($1–2 \times 10^{-4}$).

2. Nicotine-reversal by atropine could not be detected in preparations of rabbit's ileum because the motor response to nicotine persisted in the presence of atropine (10^{-7} to 10^{-4}).

3. An analogy is drawn between (*a*) atropine-induced nicotine- and vagal-reversals and (*b*) the nicotine-reversal produced by botulinum toxin (Ambache, 1951). The three phenomena appear to indicate the presence of inhibitory postganglionic neurones in the wall of the gut.

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* *Note added in proof.*—An alternative arrangement to that shown in Fig. 6 would be that the inhibitory fibres in the vagus are "through-fibres." If that were so, paralysing doses of nicotine, after blocking the peripheral synapse in the motor pathway of the vagus, should produce vagal reversal. Since this does not occur (see, for example, Bayliss and Starling, 1899, p. 143, who state explicitly that the action of the vagus is "permanently abolished" by nicotine) it may be concluded that there exists a peripheral synaptic relay, not only in the motor, but also in the inhibitory pathway of the vagus, as shown in Fig. 6.